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10/537,455	10/31/2005	Bruce R Zetter	CMC-009	6593

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GOODWIN PROCTER LLP
PATENT ADMINISTRATOR
EXCHANGE PLACE
BOSTON, MA 02109-2881

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MYERS, CARLA J	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/537,455	ZETTER ET AL.	
	Examiner	Art Unit	
	Carla Myers	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *24 August 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-24* is/are pending in the application.

4a) Of the above claim(s) *7-10 and 15-20* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-6,11-14 and 21-24* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *31 October 2005* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *8/24/07*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Applicant's election with traverse of Group II, claims 1-6, 11-14 and 21-24 (in part) in the reply filed on August 23, 2007 is acknowledged. The traversal is on the ground(s) that it would not require undue burden to search and examine each of the inventions together. This is not found persuasive because under PCT Rule 13.1 and 13.2, restriction is proper if the claimed invention do not share the same or corresponding special technical feature. As set forth in the restriction requirement of July 23, 2007, the technical feature linking the claimed inventions of thymosin β16 nucleic acids and proteins were known in the art at the time the invention was made (see Yokoyama et al) and thereby there is no special technical feature linking the claimed inventions. Additionally, a search for each of the inventions would not in fact be co-extensive with one another since there appears to be multiple thymosin β16 mRNA products. Thereby undue burden would be required to examine each of the claimed inventions together.

The requirement is still deemed proper and is therefore made FINAL.

2. Claims 1-6, 11-14 and 21-24 have been examined herein only to the extent that the claims read on the elected subject matter of methods for detecting a thymosin β16 protein. The subject matter of methods for detecting a thymosin β16 nucleic acid and claims 7-10 and 15-20 are withdrawn from consideration as being drawn to a non-elected invention.

Information Disclosure Statement

3. The information disclosure statement filed September 8, 2005 fails to comply with 37

CFR 1.98(a)(2), which requires a legible copy of each cited foreign patent document; each non-patent literature publication or that portion which caused it to be listed; and all other information or that portion which caused it to be listed. It has been placed in the application file, but the information referred to therein as reference "B1" (JP 08004717) has not been considered.

Claim Interpretation

4. It is acknowledged that the specification (para [0027]) defines thymosin β16 as being limited to "a protein having the amino acid sequence of SEQ ID NO: 1."

Priority

5. Claims 1, 3-4, 6, 11, 13, 14, 21, 23 and 24 are entitled to priority to provisional application 60/438,861, filed January 9, 2003. However, claims 2, 5, 12 and 22 are entitled to priority only to PCT/US04/00447. Provisional application '861 does not provide support for the concepts set forth in claims 2 and 5 of methods in which the sample is a nipple aspirate or the concept set forth in claims 12 and 22 of methods wherein thymosin β16 is detected by mass spectrometry. Accordingly, claims 2, 5, 12 and 22 are not entitled to priority to '861, filed January 9, 2003.

Claim Rejections - 35 USC § 112 second paragraph

6. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 3 and 6 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 3 and 6 are indefinite because the claims contain information in parentheses, i.e., "(cerebellum, medulloblastoma, astrocytoma, ependymoma, glioblastoma)," "(retinoblastoma)" and "(rhabdosarcoma)". Parentheticals make the claims indefinite because it is unclear whether the information in the parentheses has the same, less, or more weight as the rest of the claim language. For example, it is unclear as to whether the claim is intended to be limited to methods in which the cancer is retinoblastoma or methods in which the cancer is any type of eye cancer.

Claim Rejections - 35 USC § 112

7. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-6, 11-14 and 21-24 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for:

A method of diagnosing cancer in a human patient, comprising:
a) obtaining a test sample from a human patient;
b) measuring the level of thymosin β16 protein (SEQ ID NO: 1) in the test sample; and
c) comparing the level of thymosin β16 protein (SEQ ID NO: 1) in the test sample with the level of thymosin β16 protein present in a normal control sample; wherein a higher level of thymosin β16 protein in the test sample as compared to the level in the normal control sample is indicative of cancer, and wherein the sample is a prostate tissue sample and the cancer is prostate carcinoma, the sample is a lung tissue sample and the cancer is lung carcinoma, the sample is a breast tissue sample and the cancer is breast carcinoma, the sample is a thyroid tissue sample and the cancer is thyroid carcinoma, the sample is a pancreatic tissue sample and the cancer is pancreatic carcinoma, or the sample is a blood sample and the cancer is T or B cell lymphoma,

does not reasonably provide enablement for methods for diagnosing or prognosing any type of cancer in any non-human patient by assaying any biological sample for thymosin β16 protein. The specification does not enable any person skilled

in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The following factors have been considered in formulating this rejection (*In re Wands,* 858F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988): the breadth of the claims, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art, the amount of direction or guidance presented, the presence or absence of working examples of the invention and the quantity of experimentation necessary.

Breadth of the Claims:

The claims are drawn broadly to encompass methods for diagnosing cancer in a patient. While the specification (para [0041]) indicates that one example of a patient is a human patient, the claims are not limited to human patients. Rather the claims further include the diagnosis of cancer in any non-human patient, including dogs, monkeys, rats, rabbits etc.

The claims also encompass the analysis of any biological sample including blood, serum, urine, feces, saliva, sweat, ascites fluid, cerebrospinal fluid, nipple aspirates, etc for the presence or level of thymosin β16 as indicative of the occurrence or prognosis of any type of cancer.

Claims 4-6 and 21-24 further encompass prognostic evaluation of a patient based on the level of thymosin β16 protein. Thereby, the claims encompass determining the potential aggressiveness of any type of cancer, the likelihood of metastasis of any

type of cancer, prediction of the stage of any type of cancer, and determining the likelihood of survival of any type of cancer.

Nature of the Invention

The claims encompass methods for diagnosing a cancer by assaying for thymosin β16 protein. The invention is in a class of inventions which the CAFC has characterized as 'the unpredictable arts such as chemistry and biology" (Mycolgen Plant Sci., Inc. v. Monsanto Co., 243 F.3d 1316, 1330 (Federal Circuit 2001)).

Teachings in the Specification and State of the Art:

The specification (para [009]) teaches the human thymosin β16 protein consisting of SEQ ID NO: 1.

The specification (page 18 and Figure 6) further teaches the results of assays for determining the presence of thymosin β16 protein in normal human tissues. It is stated that thymosin β16 mRNA could not be detected in any of the normal tissues analyzed, including heart, brain, placenta, lung, skeletal, muscle, kidney, and pancreas tissues. However, page 37 of the specification indicates that thymosin β16 mRNA is present at low levels in prostate and ovary.

The specification (pages 37 and Figure 5) teaches the results of assays for determining the presence of thymosin β16 protein in human cancer tissues. It is disclosed that thymosin β16 protein was detectable at increased levels (as compared to normal tissues) in prostate carcinoma, lung carcinoma, breast carcinoma, thyroid carcinoma, pancreatic carcinoma and lymphoma. Results of performing EST and SAGE analysis of thymosin β16 mRNA expression in tumor tissues are also provided. The

specification teaches that thymosin β16 mRNA was detected at increased levels by EST and/or SAGE analysis in prostate carcinoma, lung carcinoma, breast carcinoma, the brain cancers of cerebellum, medulloblastoma, astrocytoma, ependymoma, and glioblastoma, retinoblastoma, rhabdosarcoma, pancreatic carcinoma, lymphoma, stomach carcinoma, and ovarian carcinoma. By Northern blotting, increased levels of thymosin β16 mRNA were detectable in prostate and breast carcinoma. And using array analysis, increased levels of thymosin β16 mRNA were detectable in prostate carcinoma. The specification does not clarify whether the thymosin β16 mRNA consisted of the mRNA of SEQ ID NO: 2 or SEQ ID NO: 3. Further, the specification does not provide any information regarding the level of protein in the brain cancers of cerebellum, medulloblastoma, astrocytoma, ependymoma, and glioblastoma, retinoblastoma, rhabdosarcoma, stomach carcinoma, and ovarian carcinoma. It is unclear as to whether thymosin β16 protein was not detectable in these cancers or whether analysis of thymosin β16 protein was not performed in these samples. In the absence of sufficient information regarding thymosin β16 protein levels in the brain cancers of cerebellum, medulloblastoma, astrocytoma, ependymoma, and glioblastoma, retinoblastoma, rhabdosarcoma, stomach carcinoma, and ovarian carcinoma, it cannot be determined if thymosin β16 protein levels are associated with the occurrence of these cancers.

Additionally, the specification teaches that thymosin β16 is expressed in human prostate cancer tumors but not in specimens of benign prostate hyperplasma (page 3).

The Predictability or Unpredictability of the Art and Degree of Experimentation:

The art of determining an association between gene expression levels and the occurrence of a cancer is highly unpredictable. Knowledge that a gene is expressed in one type of cancer does not allow one to conclude that this gene is also associated with the occurrence of all other types of cancer or with the prognosis of any type of cancer. Further, knowledge that a protein is present at higher levels in a cancer tissue does not allow one to ascertain which additional tissues or fluids will also overexpress a protein as diagnostic of cancer.

Further, knowledge that expression of a protein is expressed at higher levels in particular cancers of a human does not allow one to conclude that this protein will also be expressed at higher levels in cancers of other organisms. The specification has not established that a thymosin β16 protein consisting of SEQ ID NO: 1 is also present in a representative number of non-human organism. The post-filing date art of Banyard (Annals of the New York Academy of Sciences. 2007. 1112: 286-296) teaches the sequence of a number of thymosin β16 proteins from non-human organisms. While the thymosin β16 protein of SEQ ID NO: 1 appears to be present in monkeys, the thymosin β16 sequence is different in other non-human species of cow, pig, sheep, rat, chick, zebra-finch and quail (see Figure 3). There is no disclosure in the specification as originally filed of the occurrence of SEQ ID NO: 1 in a representative number of non-human species and overexpression of SEQ ID NO: 1 in a representative number of cancers of non-human species. In the absence of information regarding the functional properties of the thymosin β16 protein, it is unpredictable as to whether the thymosin

β16 of SEQ ID NO: 1 will also be present in other organisms will be expressed at an increased level in cancers of such organisms.

The post-filing date art corroborates the unpredictability of extrapolating the results of gene expression studies performed in humans to other mammals. Coleman (Drug Discovery Today. 2003. 8: 233-235) found that gene expression patterns between mice and humans shared some degree of similarity, but that the basic patterns of gene expression differed and that there was no general rule for predicting gene expression (page 234). Coleman concluded that '(t)he validity of mouse or other animal species as a human surrogate should not be assumed." These teachings of Coleman support the finding that there is no predictable means for determining whether the gene expression profile obtained in a human will be identical to that in the diverse genus of mammals encompassed by the claims.

It is also unpredictable as to what other types of samples may be assayed for thymosin β16 protein levels in order to diagnose cancer. Modification of gene expression may occur in all cells or may occur in only a subset of cells that are directly involved in cancer. In diseases such as cancer it is expected that an alteration in gene expression may occur only in those cells / tissues involved in the pathogenesis of cancer. The specification has not established that thymosin β16 protein is released from cancer cells and is sufficient stable in biological fluids, such as serum or urine, in order to allow for the predictable detection of an increase in the level of thymosin β16 protein as indicative of cancer. Further, the presence of a cellular or extracellular proteins in some types of biological samples, such as tears and saliva, may not be at

sufficient levels to allow for the identification of a change in gene expression. One cannot determine *a priori* which cells or other types of biological samples will show an altered gene expression that can be used to diagnose cancer. Such information can only be obtained through experimentation.

Additionally, the claims encompass prognostic evaluation of a patient based on the level of thymosin β16 protein. As such, the claims encompass determining whether a patient will develop a more aggressive type of cancer, the stage of cancer, the likelihood of cancer metastasis, the likelihood of survival, etc. However, the specification (page 3) discloses only an association between an increase level of thymosin β16 protein and the occurrence of prostate cancer. It is disclosed that while thymosin β16 protein levels are increased in prostate cancer, they are not increased in benign prostate hyperplasia (BPH). However, this disclosure is not sufficient to establish that the level of thymosin β16 protein is correlated with any type of prognosis in any type of cancer. In the absence of a clear structure-function relationship, it is unpredictable as to the effect of increased levels of thymosin β16 in cancer and the progression of cancer. Thereby, it is unpredictable as to whether the results obtained with BPH versus prostate carcinoma can be extrapolated to all types of progression and outcomes of cancer, and to all types of cancers.

Working Examples

The specification teaches an association between thymosin β16 protein levels in human prostate tissue sample and the cancer is prostate carcinoma, thymosin β16 protein levels in human lung tissue samples lung carcinoma, thymosin β16 protein

levels in human breast tissue sample and breast carcinoma, thymosin β16 protein levels in human thyroid tissue sample and thyroid carcinoma, thymosin β16 protein levels in human pancreatic tissue sample and pancreatic carcinoma, and thymosin β16 protein levels in blood samples and T or B cell lymphoma.

The specification does not provide any working examples of diagnosing cancer by assaying for SEQ ID NO: 1 in any non-human mammals.

The specification does not provide any working examples of diagnosing cancer by assaying for SEQ ID NO: 1 in non-tissue samples other than blood as diagnostic of T or B cell lymphoma.

Amount of Direction or Guidance Provided by the Specification:

The specification does not provide any specific guidance as to how to predictably identify additional organisms whose expression of SEQ ID NO: 1 is correlated with cancer. The specification does not teach the existence of SEQ ID NO: 1 in a representative number of non-human organisms. There is also no information provided on the functional activity of the protein of SEQ ID NO: 1 which would allow one to conclude that this protein will have a similar functional role in contributing to the development of cancer in other organisms.

There are also no teachings in the specification as to alternative biological samples which could be assayed to detect the presence of an increase in thymosin β16 protein as indicative of cancer.

While methods for expression profiling are known in the art, such methods provide only the general guidelines that allow researchers to randomly search for genes

whose expression may linked to cancer. The results of performing such methodology is highly unpredictable. The specification has provided only an invitation to experiment. The specification does not provide a predictable means for identifying additional organisms or biological samples which can be diagnosed for cancer by detecting thymosin β16 protein levels.

Conclusions:

Case law has established that '(t)o be enabling, the specification of a patent must teach those skilled in the art how to make and use the full scope of the claimed invention without 'undue experimentation.'" *In re Wright* 990 F.2d 1557, 1561. *In re Fisher,* 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970) it was determined that '(t)he scope of the claims must bear a reasonable correlation to the scope of enablement provided by the specification to persons of ordinary skill in the art". The amount of guidance needed to enable the invention is related to the amount of knowledge in the art as well as the predictability in the art. Furthermore, the Court in *Genetech Inc. v Novo Nordisk* 42 USPQ2d 1001 held that '(I)t is the specification, not the knowledge of one skilled in the art that must supply the novel aspects of the invention in order to constitute adequate enablement".

In the instant case, the claims do not bear a reasonable correlation to the scope of enablement because the specification teaches an association between the thymosin β16 in affected tissues and blood as indicative of particular types of cancers in humans. The specification does not teach an association between thymosin β16 protein levels and cancer in a representative number of non-human organisms or in a representative

number of the various biological samples encompassed by the claims. Further, the specification does not teach that changes in the level of thymosin β16 protein are correlated with any type of prognosis in any type of cancer, including the prognosis of survival, metastasis, development of a more aggressive form of cancer etc. In view of the unpredictability in the art, and the lack of disclosure in the specification and in the prior art and the unpredictability of the art, it would require undue experimentation for one of skill in the art to make and use the invention as broadly claimed.

Claim Rejections - 35 USC § 103

8. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1, 2, 4, 5, 11, 13, 14, 21, 23 and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zetter et al (U.S. Patent No. 5,858,681) in view of Yokoyama (DNA Research. 1996. 3: 311-320; cited in the IDS as reference "C2").

Zetter (col. 5) discloses a method for diagnosing cancer comprising: a) obtaining a nucleic acid sample from a patient; b) measuring the level of a thymosin protein in the test sample; c) comparing the level of the thymosin protein in the test sample with a control sample; wherein a higher level of thymosin protein in the test sample as compared to the level in a control sample is indicative of cancer. Regarding step c), Zetter teaches that the level of protein is compared to a base line level, particular a base line level obtained from a sample that is disease free (col. 5, lines 5-12). In particular, Zetter teaches the detection of a protein referred to therein as "thymosin β15" which consists of an amino acid sequence of SEQ ID NO: 2 (col. 2, lines 51-54; col. 3, lines 46 and 62-65). The "thymosin β15" protein of Zetter shares 85.5% identity with the presently claimed thymosin β16 protein:

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Zetter et:          1 MSDKPDLSEVEKFDRSKLKKTNTEEKNTLPSKETIQQEKECVQTS 45
                      ||||||||||| ||:||||||||||||||||||||||||  | |
thymosin β16        1 MSDKPDLSEVETFDKSKLKKTNTEEKNTLPSKETIQQEKEYNQRS 45
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Zetter does not teach diagnosing cancer by detecting the presently claimed protein thymosin β16.

However, Yokoyama teaches a NB thymosin beta protein which is identical to the presently claimed thymosin β16 protein (see Figure 2). Yokoyama reports that the NB thymosin beta protein "is expressed uniquely in human neuroblastomas" (page 318, col.

2 and Figure 1). The reference concludes that thymosin β16 "may be used as a new molecular marker for human neuroblastomas."

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Zetter so as to have detected the presence of the thymosin β16/NB thymosin beta protein in place of the "thymosin β15" protein because Yokoyama teaches that the presence of thymosin β16/NB thymosin beta is specifically associated with the occurrence of neuroblastoma. One of ordinary skill in the art would have been motivated to have modified the method of Zetter in this manner in order to have provided an effective means for diagnosing neuroblastoma in humans.

Regarding claims 2 and 5, Zetter teaches analyzing cancer tissue samples obtained from a patient (col. 2, line 67- col. 3, line 3).

Regarding claims 4, 5 and 21-24, Zetter further teaches comparing levels of thymosin protein from a test sample to a control sample, wherein a higher level of protein above a base line level indicates a poor prognosis (col. 3). Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Zetter so as to have determined the level of thymosin β16/NB thymosin beta in a test sample, to have compared this level to a level obtained from a normal patient that does not have cancer, and to have evaluated a patients prognosis of neuroblastoma based on said comparison in order to have provided additional information regarding the prognosis of a patient. It is noted that the claims have been interpreted as being inclusive of general methods for determining if a patient

has a poor prognosis, e.g., a poor outcome as a result of having cancer as compared to a control subject that does not have cancer. The claims do not require, for example, distinguishing between a more aggressive form of cancer and a less aggressive form of cancer. Rather, the claims recite only that it is a property of the presence of a high level of thymosin β16 that it is indicative of an aggressive form of cancer and therefore a poor prognosis.

Regarding claims 11 and 21, the method of Zetter modified as set forth above results in a method for detecting the level of thymosin β16/NB thymosin beta protein.

Regarding claims 13, 14, 23 and 24, Zetter teaches detecting thymosin protein by contacting a sample with a labeled antibody and detecting whether the labeled antibody is bound by the sample, thereby measuring levels of thymosin protein (see col. 3, lines 32-42; col. 6, lines36-43; col. 7, lines 15-16).

9. Claims 12 and 22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zetter et al in view of Yokoyama, as applied to claims 1, 2, 4, 5, 11, 13, 14, 21, 23 and 24 above, and further in view of Aebersold (U.S. Patent No. 6,670194).

The teachings of Zetter and Yokoyama are presented above. The combined references do not teach detecting thymosin β16/NB thymosin beta protein levels by mass spectrometry.

However, Aebersold teaches a rapid method for quantitating and analyzing proteins present in a mixture of proteins using mass spectrometry (see abstract and col. 3). Aebersold teaches that the mass spectrometry method may be used to quantitate the level of a particular protein in a biological sample, such as cell or tissue sample.

In view of the teachings of Aerbersold, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have further modified the method of Zetter so as to have measured thymosin β16/NB thymosin beta protein levels using mass spectrometry in order to have provided an equally effective means for determining the quantity of thymosin β16/NB thymosin beta protein as diagnostic of neuroblastoma.

10. Claims 1-6, 11, 13, 14, 21, 23 and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zetter et al (U.S. Patent No. 5,858,681) in view of Shou (PNAS. March 5, 2002. 99(5) 2830-2835; cited in the IDS as reference "C1"), as evidenced by GenBank Accession No. D82345 (cited in the IDS as reference "C18").

Zetter (col. 5) discloses a method for diagnosing cancer comprising: a) obtaining a nucleic acid sample from a patient; b) measuring the level of a thymosin protein in the test sample; c) comparing the level of the thymosin protein in the test sample with a control sample; wherein a higher level of thymosin protein in the test sample as compared to the level in a control sample is indicative of cancer. Regarding step c), Zetter teaches that the level of protein is compared to a base line level, particular a base line level obtained from a sample that is disease free (col. 5, lines 5-12). In particular, Zetter teaches the detection of a protein referred to therein as "thymosin β15" which consists of an amino acid sequence of SEQ ID NO: 2 (col. 2, lines 51-54; col. 3, lines 46 and 62-65). The "thymosin β15" protein of Zetter shares 85.5% identity with the presently claimed thymosin β16 protein:

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Zetter et:          1 MSDKPDLSEVEKFDRSKLKKTNTEEKNTLPSKETIQQEKECVQTS 45
                      |||||||||||  ||:|||||||||||||||||||||||||  | |
thymosin β16        1 MSDKPDLSEVETFDKSKLKKTNTEEKNTLPSKETIQQEKEYNQRS 45
```

Zetter does not teach diagnosing cancer by detecting the presently claimed protein thymosin β16.

However, Shou teaches a NB thymosin beta protein (see Table 1). As evidenced by GenBank Accession No. D82345, the NB thymosin beta protein is identical to the presently claimed thymosin β16 protein:

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NB thymosin beta:   1 MSDKPDLSEVETFDKSKLKKTNTEEKNTLPSKETIQQEKEYNQRS 45
                      |||||||||||||||||||||||||||||||||||||||||||||
thymosin β16        1 MSDKPDLSEVETFDKSKLKKTNTEEKNTLPSKETIQQEKEYNQRS 45
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Shou teaches that thymosin β16 protein/NB thymosin beta protein is expressed in primary human prostate tumors but not in control epithelia cells (page 2831, col. 2). Additionally, increased levels of thymosin β16 protein / NB thymosin beta were detected in tumorigenic benign prostatic hyperplasia cells and not in nontumorigenic BPH cells (Table 1 and abstract). Shou concludes that thymosin β16 protein / NB thymosin beta is a marker of tumorigenesis and tumor progression (Table 1, abstract and page 2833, col. 2 –2844, col. 1).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Zetter so as to have detected the presence of the thymosin β16/NB thymosin beta protein in place of the "thymosin β15" protein because Shou teaches that the presence of thymosin β16/NB

thymosin beta is specifically associated with the occurrence of prostate cancer and is indicative of progression of prostate cancer. One of ordinary skill in the art would have been motivated to have modified the method of Zetter in this manner in order to have provided an equally effective means for diagnosing prostate cancer and for evaluating the progression of prostate cancer in humans.

Regarding claims 2 and 5, Zetter teaches analyzing cancer tissue samples obtained from a patient (col. 2, line 67- col. 3, line 3).

Regarding claims 4, 5 and 21-24, Zetter further teaches comparing levels of thymosin protein from a test sample to a control sample, wherein a higher level of protein above a base line level indicates a poor prognosis (col. 3). Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Zetter so as to have determined the level of thymosin β16/NB thymosin beta in a test sample, to have compared this level to a level obtained from a normal patient that does not have cancer, and to have evaluated a patients prognosis of neuroblastoma based on said comparison in order to have provided additional information regarding the prognosis of a patient. It is noted that the claims have been interpreted as being inclusive of general methods for determining if a patient has a poor prognosis, e.g., a poor outcome as a result of having cancer as compared to a control subject that does not have cancer. The claims do not require, for example, distinguishing between a more aggressive form of cancer and a less aggressive form of cancer. Rather, the claims recite only that it is a property of the presence of a high level of thymosin β16 that it is indicative of an aggressive form of cancer and therefore a poor

prognosis. Further, Shou teaches that increased expression of thymosin β16 protein/ NB thymosin beta is correlated with progression of cancer and thereby modification of the method of Zetter so as to have detected increased expression of thymosin β16 protein/NB thymosin beta would have resulted in a method wherein detection of an increase in said protein relative to a control sample would have been indicative of a more aggressive form of prostate cancer, and therefore a poor prognosis for the patient.

Regarding claims 11 and 21, the method of Zetter modified as set forth above results in a method for detecting the level of thymosin β16/NB thymosin beta protein.

Regarding claims 13, 14, 23 and 24, Zetter teaches detecting thymosin protein by contacting a sample with a labeled antibody and detecting whether the labeled antibody is bound by the sample, thereby measuring levels of thymosin protein (see col. 3, lines 32-42; col. 6, lines36-43; col. 7, lines 15-16).

11. Claims 12 and 22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zetter et al in view of Shou, as evidenced by GenBank Accession No. D82345, as applied to claims 1-6, 11, 13, 14, 21, 23 and 24 above, and further in view of Aebersold (U.S. Patent No. 6,670194).

The teachings of Zetter and Shou are presented above. The combined references do not teach detecting thymosin β16/NB thymosin beta protein levels by mass spectrometry.

However, Aebersold teaches a rapid method for quantitating and analyzing proteins present in a mixture of proteins using mass spectrometry (see abstract and col.

3). Aebersold teaches that the mass spectrometry method may be used to quantitate the level of a particular protein in a biological sample, such as cell or tissue sample.

In view of the teachings of Aerbersold, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have further modified the method of Zetter so as to have measured thymosin β16/NB thymosin beta protein levels using mass spectrometry in order to have provided an equally effective means for determining the quantity of thymosin β16/NB thymosin beta protein as diagnostic of prostate cancer.

12. Claims 1-6, 11, 13, 14, 21, 23 and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zetter et al (U.S. Patent No. 5,858,681) in view of Chakravarti (Urology. 2000. 55: 635-638) as evidenced by Banyard (Annals of the New York Academy of Sciences. 2007. 1112: 286-296).

Zetter (col. 5) discloses a method for diagnosing cancer comprising: a) obtaining a nucleic acid sample from a patient; b) measuring the level of a thymosin protein in the test sample; c) comparing the level of the thymosin protein in the test sample with a control sample; wherein a higher level of thymosin protein in the test sample as compared to the level in a control sample is indicative of cancer. Regarding step c), Zetter teaches that the level of protein is compared to a base line level, particular a base line level obtained from a sample that is disease free (col. 5, lines 5-12). In particular, Zetter teaches the detection of a protein referred to therein as "thymosin β15" which consists of an amino acid sequence of SEQ ID NO: 2 (col. 2, lines 51-54; col. 3,

lines 46 and 62-65). The "thymosin β15" protein of Zetter shares 85.5% identity with the presently claimed thymosin β16 protein:

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Zetter et:          1 MSDKPDLSEVEKFDRSKLKKTNTEEKNTLPSKETIQQEKECVQTS 45
                      |||||||||||  ||:|||||||||||||||||||||||  | |
thymosin β16        1 MSDKPDLSEVETFDKSKLKKTNTEEKNTLPSKETIQQEKEYNQRS 45
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Zetter does not teach diagnosing cancer by detecting the presently claimed protein thymosin β16.

However, Chakravarti teaches a protein referred to therein as Tβ15. Chakravarti does not disclose the sequence of the Tβ15 protein. However, as evidenced by Banyard et al (of which the present inventors L. Hutchinson and B. Zetter are co-authors), the Tβ15 of Chakravarti appears to be identical to the presently claimed thymosin β16 protein. Specifically, Banyard states that the human β-thymosin family consists of three main groups, thymosin β4, β10 and β15 (page 286). It is stated that "in this report, we identify NB thymosin β as the human homolog of rat thymosin β15." Banyard (page 289) cites the presently applied Chakravarti reference as teaching that "A higher proportion of prostate-specific antigen (PSA) failure and bone metastasis was seen in patients with high thymosin β15 prior to therapy." In Figure 5, Banyard characterizes human thymosin β15 as having an amino acid sequence identical to presently claimed thymosin β16. Accordingly, in the absence of evidence to the contrary, it has been interpreted that the thymosin β15 protein disclosed by Chakravarti is identical to the presently claimed thymosin β16 protein.

Chakravarti teaches that Tβ15/thymosin β16 protein is expressed in primary human prostate tumors but not in normal prostatic acinar epithelium or benign hyperplastic epithelium (page 636, col. 2). Chakravarti found that increased levels of Tβ15/thymosin β16 protein (i.e., a stain of "3+") "strongly predicted the subsequent development of PSA failure with bony metastases...At 5 years of follow-up, a Tβ15 staining score of 3+ strongly predicted for PSA failure" (see page 636, col. 2). The reference also states that the level of expression of Tβ15 is correlated with clinical outcome and that Tβ15 staining intensity may provide an important marker to identify high-risk patients with moderately differentiated, clinically localized prostate cancer (see abstract).

Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Zetter so as to have detected the presence of the thymosin protein of Chakravarti (i.e., thymosin β16) in place of the thymosin protein of Zetter because Chakravarti teaches that the presence of this protein is specifically associated with the occurrence of prostate cancer and that high levels of expression of this protein strongly correlates with clinical outcome and predicts PSA failure with bony metastases.

Regarding claims 2 and 5, Zetter teaches analyzing cancer tissue samples obtained from a patient (col. 2, line 67- col. 3, line 3).

Regarding claims 4, 5 and 21-24, Zetter further teaches comparing levels of thymosin protein from a test sample to a control sample, wherein a higher level of protein above a base line level indicates a poor prognosis (col. 3). Accordingly, it would

have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Zetter so as to have determined the level of thymosin β16 in a test sample, to have compared this level to a level obtained from a normal patient that does not have cancer, and to have evaluated a patients prognosis of neuroblastoma based on said comparison in order to have provided additional information regarding the prognosis of a patient. It is noted that the claims have been interpreted as being inclusive of general methods for determining if a patient has a poor prognosis, e.g., a poor outcome as a result of having cancer as compared to a control subject that does not have cancer. The claims do not require, for example, distinguishing between a more aggressive form of cancer and a less aggressive form of cancer. Rather, the claims recite only that it is a property of the presence of a high level of thymosin β16 that it is indicative of an aggressive form of cancer and therefore a poor prognosis. Further, Chakravarti teaches that higher levels of Tβ15/thymosin β16 are correlated with PSA failure and bony metastasis. Thereby modification of the method of Zetter so as to have detected increased expression of the Tβ15/thymosin β16 protein of Chakravarti would have resulted in a method wherein detection of a high level of expression of said protein relative to a control sample would have been indicative of a more aggressive form of prostate cancer, and therefore a poor prognosis for the patient.

Regarding claims 11 and 21, the method of Zetter modified as set forth above results in a method for detecting the level of Tβ15/thymosin β16 protein.

Regarding claims 13, 14, 23 and 24, Zetter teaches detecting thymosin protein by contacting a sample with a labeled antibody and detecting whether the labeled

antibody is bound by the sample, thereby measuring levels of thymosin protein (see col. 3, lines 32-42; col. 6, lines36-43; col. 7, lines 15-16).

13. Claims 12 and 22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zetter et al in view of Chakravarti, as evidenced by Banyard, as applied to claims 1-6, 11, 13, 14, 21, 23 and 24 above, and further in view of Aebersold (U.S. Patent No. 6,670194).

The teachings of Zetter and Chakravarti are presented above. The combined references do not teach detecting thymosin β16 protein levels by mass spectrometry.

However, Aebersold teaches a rapid method for quantitating and analyzing proteins present in a mixture of proteins using mass spectrometry (see abstract and col. 3). Aebersold teaches that the mass spectrometry method may be used to quantitate the level of a particular protein in a biological sample, such as cell or tissue sample.

In view of the teachings of Aerbersold, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have further modified the method of Zetter so as to have measured thymosin β16 protein levels using mass spectrometry in order to have provided an equally effective means for determining the quantity of thymosin β16 protein as diagnostic of prostate cancer.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Carla Myers whose telephone number is 571-272-0747. The examiner can normally be reached on Monday-Thursday (6:30-5:00).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on 571-272-0735. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Carla Myers/
Primary Examiner, Art Unit 1634